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Research Article

Toxicological Evaluation of Resistant Dextrin Preparations Derived from Tapioca and Corn Starch

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Abstract

A series of toxicity studies were performed to determine the safety of resistant dextrin preparations derived from tapioca starch (RD-tapioca; FiberSMART® Soluble Tapioca Fiber) and corn starch (RD-corn; FiberSMART® Soluble Tapioca Fiber). In the bacterial reverse mutation test, the mutagenicity of RD-corn and RD-tapioca was evaluated in *Salmonella typhimurium* TA97, TA98, TA100, TA102, and TA1535. RD-corn and RD-tapioca doses up to 5,000 µg/plate did not increase the number of revertant colonies, and, thus, were not mutagenic. In the acute toxicity study, SD rats received a single dose of control, and up to 20 g/kg bw RD-tapioca and RD-corn. The mean lethal does (LD_{50}) was above 20 g/kg bw for both RD-tapioca and RD-corn for male and female rats. In the subchronic toxicity study, Sprague-Dawley (SD) rats consumed 0, 1,250, 2,500, or 5,000 mg/kg bw/day RD-tapioca for 90 days. The No Observed Adverse Effect Level (NOAEL) was higher than 5,000 mg/kg bw/day, the highest dose tested, for male and female rats. The mutagenicity, acute toxicity and subchronic studies demonstrated the safety of RD-tapioca.

Keywords: *Resistant Dextrin; FiberSMART® Soluble Tapioca Fiber; FiberSMART® Soluble Corn Fiber; Mutagenicity; Acute Toxicity; Subchronic Toxicity*

Research Highlights

- RD-tapioca and RD-corn were not mutagenic.
- Mean lethal dose for RD-tapioca and RD-corn was higher than 20 g/kg bw in Sprague-Dawley (SD) rats.
- RD-tapioca NOAEL was 5,000 mg/kg bw/day, the highest level tested, in SD rats.

Introduction

Resistant dextrin is an indigestible and soluble dietary fiber produced from dextrin. Dextrin is produced by partial hydrolyzation of starch using heat and/or food-grade acids. Depending on the reaction conditions, dextrinization, a dry roasting process, generates products with varied properties, such as molecular weight, color, viscosity, cold-water solubility, sugar content, and stability. It generally reduces the molecular weight and produces new glycosidic linkages. The dextrin is then purified, producing resistant dextrin [1].

Compared to starches and maltodextrins, which contain only digestible glycosidic linkages, dextrin contains both digestible and indigestible glycosidic linkages. Due to these indigestible linkages, dextrin is not hydrolyzed by human digestive enzymes and passes unchanged through the upper portion of the gastrointestinal tract. The undigested dextrin is hydrolyzed in the colon by bacterial flora, producing free fatty acids that are used for energy [1].

Although resistant dextrin preparations derived from corn starch (RD-corn) and wheat starch (RD-wheat) have undergone toxicological evaluation, the preparation derived from tapioca starch (RD-tapioca) has never been evaluated. This is the first study to describe the safety of RD-tapioca while confirming the safety of RD-corn.

Materials and Methods

Materials

Test article

Resistant dextrin (RD) from corn (RD-corn, or FiberSMART® Soluble Corn Fiber) and tapioca (RD-tapioca or FiberSMART® Soluble Tapioca Fiber) were supplied by Anderson Global Group, Irvine, California, USA.

Microorganisms, cell lines, and cultures

For the mutagenicity study, *Salmonella typhimurium* strains (TA97, TA98, TA100, TA102, and TA1535) were obtained from Molecular Toxicology, Inc. (Boone, North Carolina, USA) and maintained as frozen stocks at -80 ± 3°C.

Animals

For the acute toxicity study, 6-week-old Specific Pathogen Free Sprague-Dawley (SPF SD) rats were housed in cages under hygienic conditions in a controlled environment with a 12-hour light/dark cycle at 23 ± 3°C and 40 - 60% humidity. The rats consumed a commercial standard rat cube diet and water *ad libitum*. The use of the laboratory animals was in accordance with the Guidelines of Animal Care.

For the subchronic toxicity study, SPF SD rats weighing 115 ± 15g were obtained from Jinan Pengyue Experimental Animal Breeding Co. Ltd. (Jinan, China). The rats were housed in cages under hygienic conditions in a controlled environment with a 12-hour light/dark cycle at 23 ± 3°C and 40 - 70% humidity for 7 days before the study. The use of the laboratory animals was in accordance with the Guidelines of Animal Care and Use of Laboratory Animals from the Association of Laboratory Animal Science and the Center for Laboratory Animal Science at Yantai University (Yantai, China).

Experimental design

The study protocols were adopted from the Organization for Economic Co-operation and Development (OECD) guidelines for safety studies: bacterial reverse mutation study - OECD 471; acute oral toxicity study in rats - FDA Redbook 2000; and 90-day oral toxicity studies in rats - OECD 408.

Bacterial reverse mutation test

The mutagenicity of resistant dextrin (RD)-corn and RD-tapioca was evaluated in *Salmonella typhimurium* strains (TA97, TA98, TA100, TA102, and TA1535) using the plate incorporation method. The concentrations for the test were selected based on a preliminary study demonstrating that up to 5,000 µg/plate of RD-corn and RD-tapioca did not show any antibacterial activity. TA97, TA98, TA100, TA102, and TA1535 were treated with 0 (solvent control), 1,250, 2,500, or 5,000 µg/plate of RD-corn or RD tapioca in the absence or presence of

an exogenous metabolic activation system (S9). Triplicate plates were prepared for each concentration. All plates were incubated at 37°C for 72 hours, and then the number of revertant colonies were determined.

The positive controls in the absence of S9 were 4-nitro-o-phenylenediamine (NPD), daunomycin (DAM), sodium azide (NaN_3) and methyl methanesulfonate (MMS). In the presence of S9, the positive controls were 2-aminofluorene (2-AF), 1,8-dihydroxyanthraquinone (1,8-DT), and 2-aminoanthracene (2-AA).

Acute toxicity study

The toxicity of a single dose of RD-tapioca and RD-corn was evaluated in male and female SD rats. Based on their body weight taken before treatment, the rats were randomized into three groups (n = 5/sex/group): control (purified water), 20 g/kg bw RD-corn, or 20 g/kg bw RD-tapioca. During the 14-day observation period, clinical signs of toxicity, mortality, and morbidity were monitored twice daily. Body weight was measured at pre-test, weekly thereafter, and at sacrifice. Food consumption was also observed. At the end of the treatment, body weight and main organ weights, including liver, kidneys, spleen, heart, brain, and lungs, were measured and the organ coefficient (organ/body weight ratio) was reported. The tissues were examined, and gross lesions were examined microscopically. If treatment-related effects were observed in the tissues, they were examined microscopically.

Subchronic toxicity study

The subchronic toxicity of RD-tapioca was assessed in SPF SD rats. The rats were randomized into four groups (n = 11/sex/group) based on pre-test body weight. They consumed 0, 1,250, 2,500, or 5,000 mg/kg bw/day resistant dextrin, respectively, for 90 days. Clinical signs of toxicity were observed twice daily. Body weight was measured at pre-test, weekly thereafter, and at sacrifice after fasting. Feed consumption was assessed weekly. Pre-test ophthalmic examination was administered to each rat. At termination, the control and 5,000 mg/kg bw/day (high-dose) group rats underwent ophthalmic examination. If treatment-related changes in the eye were observed, ophthalmological examinations were performed on all rats.

During the last week of the study, urine from the surviving rats was collected and analyzed for appearance, color, volume, pH, specific gravity, leukocytes, nitrite, protein, glucose, ketones, urobilinogen and bilirubin.

To collect the blood samples, rats were fasted overnight and then anesthetized with CO_2 gas. The blood was collected into separate tubes containing EDTA. The following hematological parameters were evaluated: hemoglobin (Hb), hematocrit (HCT), red blood cells (RBC), white blood count (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), reticulocyte (RET%), neutrophils (NEUT%), lymphocytes (LYMPH%), and monocytes (MONO%).

Blood samples were centrifuged to separate the blood plasma. The following plasma biochemistry parameters were analyzed: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), bilirubin (TBIL), glucose (GLU), cholesterol (CHOL), creatinine (CREA), urea nitrogen (UREA), triglycerides (TG), phosphorous (P), calcium (Ca), sodium (Na), potassium (K), and chloride (Cl).

At the end of treatment, surviving rats were fasted overnight. The rats were weighed prior to exsanguination. External and internal gross pathological examinations were then assessed.

The following organs and tissues were collected, preserved in 10% neutral buffered formalin, and examined macroscopically: adrenals, aorta, bone (femur), bone marrow (sternum), brain, cecum, colon, uterus, duodenum, epididymis, esophagus, harderian gland, heart,

ileum, jejunum, kidneys, liver, lung, mandibular lymph nodes, mesenteric lymph nodes, mammary glands, nasal turbinates, ovaries, pancreas, pituitary, prostate, rectum, salivary gland, sciatic nerve, seminal vesicle, skeletal muscle, skin, spinal cord (cervical, mid-thoracic, and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, vagina, and all tissues showing abnormality. All gross lesions were further examined microscopically.

The following organs were weighed wet: liver, kidneys, adrenals, spleen, heart, lung, uterus/epididymides, thymus, brain, testes/ovaries, and thyroid/parathyroid (postfixed). Relative organ weights against fasting body weight were calculated. In the control and high-dose group rats, all the mentioned organs were examined microscopically. If treatment-related effects were observed, organs of the next lower dose level group were examined. Successive examination of the next lower dose level continued until no effects were observed. All tissues from animals that died prematurely or were sacrificed during the study were examined microscopically.

Statistical analysis

Mutagenicity and acute tox: SPSS 11.5 software for Windows was used to perform all analyses. To compare the test dose and control group data, one-way ANOVA with Dunnett's post-hoc test was used. A P-value less than 0.05 was considered statistically significant.

For the subchronic toxicity study, quantitative data were evaluated using the LEVENE test. If homogeneity of variance was found ($P > 0.05$), one-way ANOVA was used. Kruskal-Wallis H tests were used if heterogeneity of variance was found ($P \leq 0.05$). If ANOVA was significant ($P \leq 0.05$), then Dunnett's test was used for pairwise comparisons. Statistical analysis was completed if ANOVA was not significant ($P > 0.05$). If Kruskal-Wallis H tests were significant ($P \leq 0.05$), then Mann-Whitney U tests were used. Statistical analysis was completed if Kruskal-Wallis H tests were not significant ($P > 0.05$). Ordinal categories data were analyzed by Kruskal-Wallis H tests. Mann-Whitney U tests were used if significant differences ($P \leq 0.05$) were found. Fisher's exact probabilities test was used to analyze binomial categories data. Fisher's exact probabilities test was used for pairwise comparisons if significant differences ($P \leq 0.05$) were found.

Results

Bacterial reverse mutation test

The test substance was considered mutagenic if the number of revertant colonies in the test dose groups was more than two-fold or increased in a dose-dependent manner compared to the control group in at least one strain in the absence or presence of S9. Table 1 presents the results of bacterial reverse mutation test conducted on RD-tapioca. At any RD-tapioca doses in any tester strains, there were no increases in revertant frequencies compared to the vehicle control group in the absence or presence of S9. In addition, the tests conducted on RD-corn also did not show any increases in revertant frequencies compared to the control group in the absence or presence of S9 (data not shown). The positive controls for each tester strain induced obvious increases in the number of revertant colonies compared to the vehicle control. The data indicated that RD-corn and RD-tapioca were not mutagenic under the conditions of this test.

	Dose (μg/plate) TA97		Mean Revertant Colony Counts Per Plate				
			TA98	TA100	TA102	TA1535	
-S9	Vehicle control		110.7 ± 10.7	34.3 ± 5.9	203.7 ± 15.6	261.3 ± 21.4	63.0 ± 24.6
	RD-corn	5,000	123.7 ± 9.6	26.3 ± 8.5	177.3 ± 22.0	248.7 ± 19.7	70.0 ± 31.2
		2,500	103.7 ± 18.1	30.0 ± 5.0	186.7 ± 3.8	275.0 ± 28.2	78.0 ± 24.3
		1,250	97.3 ± 11.2	23.3 ± 5.5	201.0 ± 34.4	288.0 ± 27.2	88.7 ± 39.9
	RD-tapioca	5,000	108.7 ± 20.6	27.7 ± 2.1	206.7 ± 25.0	270.3 ± 24.5	94.3 ± 11.2
		2,500	106.0 ± 8.9	31.3 ± 4.0	204.3 ± 19.8	289.7 ± 45.5	111.0 ± 25.6
		1,250	112.7 ± 17.1	30.0 ± 4.0	182.7 ± 39.1	278.0 ± 24.00	71.3 ± 15.6
	NPD	20	1,084.0 ± 113.5**	—	—	—	—
	DAM	10	—	861.7 ± 199.9**	—	—	—
	NaN_3	1.5	—	—	1,168.3 ± 198.3**	—	866.3 ± 45.7**
	MMS	2	—	—	—	926.3 ± 169.5**	—

+S9	Vehicle control		152.7 ± 7.1	42.0 ± 5.2	243.0 ± 20.4	309.0 ± 23.6	96.0 ± 25.9
	RD-corn	5,000	128.3 ± 6.7	45.7 ± 5.9	218.0 ± 6.9	289.7 ± 10.5	115.0 ± 12.0
		2,500	147.0 ± 13.1	40.0 ± 6.1	257.3 ± 46.1	311.0 ± 22.6	113.3 ± 23.4
		1,250	137.0 ± 4.0	42.0 ± 3.5	227.7 ± 9.3	318.0 ± 2.0	124.0 ± 10.1
	RD-tapioca	5,000	127.7 ± 14.8	38.3 ± 2.3	219.7 ± 31.9	261.7 ± 28.7	148.7 ± 26.3
		2,500	133.0 ± 8.2	24.3 ± 2.1	209.7 ± 10.6	298.3 ± 25.9	143.3 ± 22.0
		1,250	161.3 ± 12.2	43.7 ± 10.6	192.3 ± 8.3	300.7 ± 9.3	95.0 ± 1.7
	2-AF	20	836.3 ± 24.0**	958.0 ± 48.5**	1,032.3 ± 37.6**	—	—
	1,8-DT	50	—	—	—	505.0 ± 39.3**	—
	2-AA	5	—	—	—	—	438.7 ± 22.1**

Table 1: *Bacterial mutation assay results for RD-tapioca.*

Abbreviations: 1,8-DT = 1,8-Dihydroxyanthraquinone; 2-AA = 2-Aminoanthracene; 2-AF = 2-Aminofluorene; DAM = Daunomycin; MMS = Methyl Methanesulfonate; NaN_3 = Sodium Azide; NPD = 4-Nitro-o-Phenylenediamine.

***: P < 0.01, compared with vehicle control.*

Acute toxicity study

All rats survived to the end of the study and appeared healthy throughout. In the two test groups (RD-corn and RD-tapioca), hypoactivity and piloerection were reported after administration, but disappeared within 4 hours. There were no other obvious abnormal clinical signs in any of the groups. No body changes were observed between the groups. No significant differences were observed for food consumption and organ coefficients. During the macroscopic examination, no treatment-related effects of RD-corn and RD-Tapioca were found in the tissues. Based on the results, the mean lethal dose (LD_{50}) for RD-corn and RD-Tapioca was above 20 g/kg bw, the highest dose tested.

Subchronic toxicity study

Clinical observation, feed consumption, and body weights

At any dose level, no clinical signs of toxicity or mortality were observed. No differences in body weight (Figure 1 and 2) and feed consumption were observed between the groups. Urinalysis did not show any treatment-related adverse effects (data not shown).

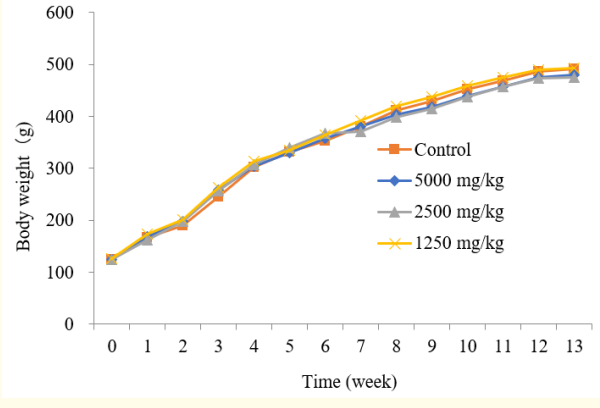

Figure 1: *Effect of RD-tapioca on body weight in male rats.*

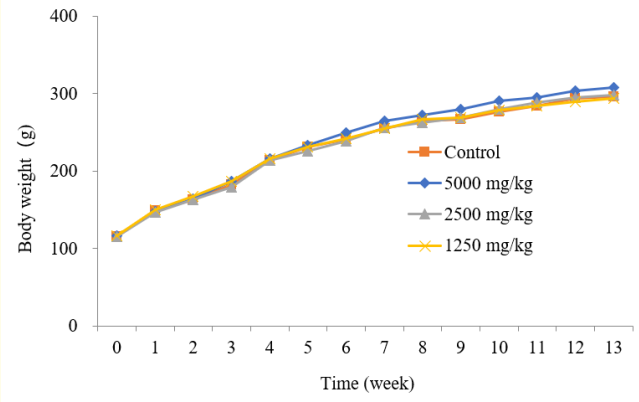

Figure 2: *Effect of RD-tapioca on body weight in female rats.*

Hematology

In female rats, there were no consistent, statistically significant, dose-dependent, or treatment-related adverse effects on any of the hematology parameters (Table 2). In male rats in the 5,000 mg/kg bw/day group, PLT was slightly increased compared to the control group (1,091.7 ± 109.9 vs. 991.0 ± 103.6 x 10^9/L, P < 0.05). However, these changes were not considered of toxicological concern because they did not occur in both sexes and were within the laboratory's historical normal range of controls.

Parameter	Male (mg/kg bw/day)				Female (mg/kg bw/day)			
	Control	1,250	2,500	5,000	Control	1,250	2,500	5,000
WBC (x10^9/L)	4.77 ± 0.85	4.32 ± 0.86	4.10 ± 0.72	4.18 ± 0.85	3.06 ± 0.90	3.13 ± 0.87	3.03 ± 0.57	2.99 ± 0.47
Hb (g/L)	15.7 ± 0.9	16.2 ± 0.8	16.1 ± 0.5	16.5 ± 1.0	15.0 ± 1.1	15.0 ± 0.5	14.9 ± 1.0	15.1 ± 0.7
HCT (%)	48.5 ± 2.8	50.1 ± 2.7	49.8 ± 1.6	50.7 ± 3.0	45.9 ± 3.8	43.5 ± 9.5	45.5 ± 3.3	46.6 ± 2.2
RBC (x10^{12}/L)	9.41 ± 0.63	9.75 ± 0.46	9.68 ± 0.31	9.81 ± 0.52	8.46 ± 0.61	8.00 ± 1.79	8.53 ± 0.67	8.59 ± 0.44
MCV (fL)	51.6 ± 1.66	51.41 ± 0.81	51.79 ± 0.81	51.65 ± 1.19	54.18 ± 1.02	54.48 ± 1.42	53.37 ± 1.65	54.21 ± 1.54
MCH (Pg)	16.7 ± 0.53	16.60 ± 0.41	16.58 ± 0.24	16.70 ± 0.50	17.75 ± 0.46	17.65 ± 0.52	17.45 ± 0.63	17.53 ± 0.63
MCHC (g/L)	32.3 ± 0.49	32.29 ± 0.44	32.11 ± 0.25	32.17 ± 0.26	32.75 ± 0.71	32.41 ± 0.74	32.66 ± 0.33	32.35 ± 0.84
PLT (x10^9/L)	991.0 ± 103.6	1,019.6 ± 90.0	1,083.2 ± 135.2	1,091.7 ± 109.9*	850.9 ± 167.5	858.8 ± 104.8	956.6 ± 143.6	983.7 ± 134.3
NEUT (%)	17.5 ± 7.3	20.1 ± 4.4	19.2 ± 5.3	16.2 ± 4.9	14.9 ± 11.0	18.2 ± 10.2	19.3 ± 7.0	16.8 ± 10.4
LYMPH (%)	74.2 ± 8.3	70.9 ± 5.8	72.0 ± 5.8	75.7 ± 5.6	76.1 ± 13.5	73.2 ± 10.2	71.5 ± 9.2	73.7 ± 12.1
MONO (%)	6.37 ± 2.34	6.57 ± 1.51	5.85 ± 0.99	6.15 ± 1.25	7.17 ± 2.30	7.11 ± 1.85	7.22 ± 3.02	7.57 ± 1.82
RET (%)	2.76 ± 0.37	2.45 ± 0.38	2.66 ± 0.27	2.57 ± 0.24	2.65 ± 0.72	3.00 ± 0.87	2.73 ± 0.45	2.97 ± 0.96

Table 2: *Hematologic findings in male and female rats treated with RD-tapioca.*

Values were mean ± SD.

Hb = Hemoglobin; HCT = Hematocrit; LYMPH = Lymphocytes; MCH = Mean Corpuscular Hemoglobin; MCHC = Mean Corpuscular Hemoglobin Concentration; MCV = Mean Corpuscular Volume; MONO = Monocytes; NEUT = Neutrophils; PLT = Platelet Count; RBC = Red Blood Cell; RET = Reticulocytes; WBC = White Blood Count.

**: P < 0.05 vs. control group.*

Plasma biochemistry

No clinically significant abnormalities were found in any of RD-tapioca treated groups (Table 3). In females, the total protein level was slightly decreased in the 1,250 mg/kg bw/day group (62.1 ± 5.6 *vs.* 67.5 ± 4.7 g/L, P < 0.05) and the cholesterol level was decreased in the 2,500 mg/kg bw/day group (1.92 ± 0.19 *vs.* 2.12 ± 0.23 mmol/L, P < 0.05). The male 1,250 mg/kg bw/day group had increased P level (3.12 ± 0.31 *vs.* 2.83 ± 0.32 mmol/L, P < 0.05). Increased creatine level was observed in the male 2,500 mg/kg bw/day group (46.6 ± 6.0 *vs.* 41.2 ± 6.0 µmol/L, P < 0.05). Chloride level was decreased in the male 5,000 mg/kg bw/day group (103.5 ± 0.97 *vs.* 105.1 ± 1.44 mmol/L, P < 0.05). However, these changes were not considered of toxicological significance since they did not occur in both sexes, were not dose-dependent, and were within the laboratory's historical normal range of controls.

Parameters	Male (mg/kg bw/day)				Female (mg/kg bw/day)			
	Control	1,250	2,500	5,000	Control	1,250	2,500	5,000
TP (g/L)	62.2 ± 6.6	65.9 ± 5.0	59.6 ± 5.9	62.2 ± 7.4	67.5 ± 4.7	62.1 ± 5.6*	68.2 ± 4.3	65.7 ± 7.5
ALB (g/L)	33.4 ± 3.5	32.8 ± 4.7	36.2 ± 3.9	30.0 ± 4.9	39.5 ± 4.9	42.2 ± 3.6	36.1 ± 4.7	38.5 ± 5.4
ALT (U/L)	36.6 ± 5.2	37.1 ± 5.9	35.3 ± 8.5	39.3 ± 8.9	32.8 ± 5.3	33.9 ± 4.7	31.7 ± 5.3	31.1 ± 4.9
AST (U/L)	122.7 ± 15.0	129.6 ± 14.3	115.6 ± 16.5	131.6 ± 16.2	112.2 ± 18.1	106.9 ± 18.9	116.2 ± 18.7	117.0 ± 12.4
ALP (U/L)	86.4 ± 14.7	89.8 ± 16.0	92.4 ± 6.1	85.1 ± 13.9	52.2 ± 14.1	51.9 ± 10.3	54.7 ± 12.8	49.6 ± 8.2
CREA (µmol/L)	41.2 ± 6.0	43.3 ± 3.7	46.6 ± 6.0*	44.7 ± 6.4	43.0 ± 6.3	44.0 ± 4.0	46.6 ± 4.9	38.9 ± 5.8
GLU (mmol/L)	7.88 ± 1.36	7.61 ± 0.90	8.12 ± 1.57	8.02 ± 0.86	6.77 ± 0.79	6.81 ± 0.89	6.68 ± 0.90	6.89 ± 0.73
Ca (mmol/L)	2.63 ± 0.26	2.69 ± 0.25	2.79 ± 0.41	2.77 ± 0.33	2.52 ± 0.16	2.59 ± 0.19	2.65 ± 0.24	2.67 ± 0.26
P (mmol/L)	2.83 ± 0.32	3.12 ± 0.31*	3.07 ± 0.28	2.95 ± 0.30	2.18 ± 0.23	1.97 ± 0.26	2.33 ± 0.33	2.24 ± 0.32
TG (mmol/L)	0.63 ± 0.17	0.59 ± 0.12	0.63 ± 0.13	0.66 ± 0.11	0.58 ± 0.14	0.52 ± 0.15	0.58 ± 0.16	0.57 ± 0.14
CHOL (mmol/L)	1.85 ± 0.33	1.69 ± 0.29	1.72 ± 0.34	1.69 ± 0.39	2.12 ± 0.23	2.09 ± 0.35	1.92 ± 0.19*	1.96 ± 0.50
UREA (mmol/L)	7.49 ± 0.91	7.31 ± 0.85	7.38 ± 1.05	7.93 ± 0.83	8.41 ± 1.71	9.02 ± 0.44	8.44 ± 1.58	8.96 ± 1.40
BIL (µmol/L)	0.76 ± 0.15	0.79 ± 0.11	0.71 ± 0.14	0.66 ± 0.13	0.86 ± 0.16	0.80 ± 0.16	0.79 ± 0.13	0.84 ± 0.10
Na (mmol/L)	139.4 ± 1.3	139.7 ± 1.6	138.7 ± 1.3	139.5 ± 1.7	137.3 ± 2.5	138.0 ± 1.6	138.5 ± 1.1	136.7 ± 2.2
K (mmol/L)	5.51 ± 0.29	5.49 ± 0.39	5.49 ± 0.45	5.55 ± 0.71	4.58 ± 0.41	4.61 ± 0.35	4.71 ± 0.41	4.77 ± 0.43
Cl (mmol/L)	105.1 ± 1.4	104.9 ± 1.6	104.9 ± 1.7	103.5 ± 1.0*	103.8 ± 1.8	104.1 ± 1.6	102.9 ± 2.2	103.6 ± 2.0

Table 3: *Clinical biochemistry in male and female rats treated with RD-tapioca.*

Values were mean ± SD.

ALB = Albumin; ALP = Alkaline Phosphatase; ALT = Alanine Aminotransferase; AST = Aspartate Aminotransferase; BIL = Bilirubin; CHOL = Cholesterol; Cl = Chloride; CREA = Creatinine; GLU = Glucose; K = Potassium; Na = Sodium; TG = Triglycerides; TP = Total Protein; UREA = Urea Nitrogen.

**P < 0.05 vs. control group.*

Terminal organ weights, organ/body weight ratio, and histopathological examination

No clinically significant abnormalities were found in any RD-tapioca treated groups (Table 4 and 5). Females in the 5,000 mg/kg bw/day group had increased absolute organ weights of lung (1.55 ± 0.31 *vs.* 1.30 ± 0.13 g, P < 0.05) and thyroid/parathyroid (0.031 ± 0.007 *vs.* 0.024 ± 0.006 g, P < 0.05). Additionally, the relative weight (mean organ-to-terminal body weight ratios) of the thyroid/parathyroid was increased (0.010 ± 0.002 *vs.* 0.008 ± 0.002%, P < 0.05). In the male 2,500 mg/kg bw/day group, the absolute and relative weights of the pituitary were increased compared to the control (0.009 ± 0.003 *vs.* 0.012 ± 0.003 g; 0.019 ± 0.006 *vs.* 0.0025 ± 0.0005%; P < 0.05; respectively). These effects were not considered of toxicological significance since the changes did not occur in both sexes, were not dose-dependent, and were within the laboratory's historical normal range of controls.

Parameters (g)	Male (mg/kg bw/day)				Female (mg/kg bw/day)			
	Control	1,250	2,500	5,000	Control	1,250	2,500	5,000
Liver	11.33 ± 1.29	11.87 ± 0.94	10.79 ± 1.22	10.72 ± 1.96	7.23 ± 0.73	7.46 ± 0.86	7.62 ± 0.61	7.91 ± 1.15
Kidneys	3.00 ± 0.23	3.26 ± 0.30	2.90 ± 0.24	2.96 ± 0.57	1.78 ± 0.14	1.76 ± 0.16	1.86 ± 0.18	1.85 ± 0.23
Adrenals	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.01	0.05 ± 0.02	0.07 ± 0.01	0.07 ± 0.02	0.07 ± 0.02	0.08 ± 0.01
Spleen	0.69 ± 0.08	0.76 ± 0.12	0.72 ± 0.09	0.63 ± 0.09	0.48 ± 0.05	0.52 ± 0.06	0.52 ± 0.07	0.53 ± 0.11
Heart	1.45 ± 0.17	1.40 ± 0.22	1.32 ± 0.10	1.37 ± 0.23	0.90 ± 0.11	0.95 ± 0.09	1.01 ± 0.16	0.95 ± 0.08
Lung	1.69 ± 0.22	1.77 ± 0.26	1.81 ± 0.25	1.62 ± 0.35	1.30 ± 0.13	1.35 ± 0.14	1.49 ± 0.27	1.55 ± 0.31*
Uterus					0.55 ± 0.18	0.64 ± 0.17	0.58 ± 0.15	0.70 ± 0.15
Ovaries					0.15 ± 0.04	0.15 ± 0.06	0.17 ± 0.02	0.17 ± 0.02
Testes	3.29 ± 0.27	3.42 ± 0.46	3.12 ± 0.25	3.21 ± 0.29				
Epididymides	1.31 ± 0.16	1.30 ± 0.09	1.35 ± 0.15	1.25 ± 0.14				
Thymus	0.43 ± 0.09	0.43 ± 0.08	0.47 ± 0.18	0.39 ± 0.09	0.37 ± 0.09	0.37 ± 0.04	0.40 ± 0.12	0.41 ± 0.12
Brain	1.86 ± 0.47	1.93 ± 0.15	1.78 ± 0.25	1.93 ± 0.09	1.69 ± 0.40	1.79 ± 0.14	1.80 ± 0.30	1.82 ± 0.30
Pituitary	0.012 ± 0.003	0.012 ± 0.002	0.009 ± 0.003*	0.011 ± 0.003	0.014 ± 0.003	0.013 ± 0.002	0.015 ± 0.004	0.016 ± 0.003
Thyroid/ parathyroid	0.036 ± 0.008	0.030 ± 0.007	0.037 ± 0.013	0.037 ± 0.013	0.024 ± 0.006	0.027 ± 0.011	0.030 ± 0.009	0.031 ± 0.007*

Table 4: *Mean absolute organ weights in male and female rats treated with RD-tapioca.*

Values were mean ± SD.

**: P < 0.05 vs. control group.*

Parameters (%)	Male (mg/kg bw/day)				Female (mg/kg bw/day)			
	Control	1,250	2,500	5,000	Control	1,250	2,500	5,000
Liver	2.31 ± 0.18	2.42 ± 0.23	2.29 ± 0.20	2.23 ± 0.22	2.45 ± 0.21	2.55 ± 0.17	2.57 ± 0.16	2.57 ± 0.32
Kidneys	0.61 ± 0.04	0.66 ± 0.08	0.62 ± 0.05	0.61 ± 0.06	0.61 ± 0.06	0.60 ± 0.03	0.63 ± 0.04	0.60 ± 0.06
Adrenals	0.0115 ± 0.0036	0.0118 ± 0.0033	0.0128 ± 0.0031	0.0112 ± 0.0038	0.023 ± 0.005	0.024 ± 0.008	0.023 ± 0.008	0.025 ± 0.005
Spleen	0.14 ± 0.01	0.15 ± 0.03	0.15 ± 0.02	0.13 ± 0.01	0.16 ± 0.02	0.18 ± 0.03	0.17 ± 0.03	0.17 ± 0.04
Heart	0.29 ± 0.03	0.29 ± 0.05	0.28 ± 0.02	0.29 ± 0.04	0.31 ± 0.02	0.33 ± 0.02	0.34 ± 0.04	0.31 ± 0.03
Lung	0.35 ± 0.04	0.36 ± 0.06	0.39 ± 0.06	0.34 ± 0.07	0.44 ± 0.05	0.46 ± 0.05	0.50 ± 0.10	0.50 ± 0.09
Uterus					0.19 ± 0.06	0.22 ± 0.07	0.20 ± 0.05	0.23 ± 0.06
Ovaries					0.05 ± 0.02	0.49 ± 0.016	0.06 ± 0.01	0.06 ± 0.01
Testes	0.67 ± 0.04	0.70 ± 0.11	0.66 ± 0.06	0.67 ± 0.08				
Epididymides	0.27 ± 0.03	0.26 ± 0.03	0.29 ± 0.03	0.26 ± 0.03				
Thymus	0.09 ± 0.02	0.09 ± 0.02	0.10 ± 0.04	0.08 ± 0.02	0.13 ± 0.04	0.13 ± 0.02	0.13 ± 0.04	0.13 ± 0.04
Brain	0.38 ± 0.09	0.39 ± 0.03	0.38 ± 0.06	0.41 ± 0.04	0.57 ± 0.12	0.62 ± 0.05	0.61 ± 0.11	0.60 ± 0.12
Pituitary	0.0025 ± 0.0005	0.0025 ± 0.0004	0.0019 ± 0.0006*	0.0023 ± 0.0004	0.005 ± 0.001	0.005 ± 0.001	0.005 ± 0.001	0.005 ± 0.001
Thyroid/ parathyroid	0.0074 ± 0.0017	0.0061 ± 0.0015	0.0078 ± 0.0029	0.0077 ± 0.0027	0.008 ± 0.002	0.009 ± 0.003	0.010 ± 0.003	0.010 ± 0.002*

Table 5: *Mean relative organ weights in male and female rats treated with RD-tapioca.*

Values were mean ± SD.

**P < 0.05 vs. control group.*

Histopathological assessment found no RD-tapioca-related changes in the 5,000 mg/kg bw/day groups (Table 6). In the heart, inflammatory cell infiltration was observed in a female in the 5,000 mg/kg bw/day group and a male in the control group. The liver of a female in the 5,000 mg/kg bw/day group and males in the control and 5,000 mg/kg bw/day groups had inflammatory cell infiltration. Inflammatory cell infiltration in the kidneys was found in a control female and a 5,000 mg/kg bw/day male. Both the control and 5,000 mg/kg bw/day groups of both sexes had inflammatory cell infiltration in the lungs. However, all the observed histopathological findings were minimal, and not considered toxicologically significant. In addition, no caecal enlargement effect was observed in the high dose groups of both sexes.

Organ/Findings	Male (mg/kg bw/day)		Female (mg/kg bw/day)	
	Control	5,000	Control	5,000
Heart				
Inflammatory cell infiltration, focal	0	1(±)	1(±)	0
Liver				
Inflammatory cell infiltration, focal	0	1(±)	1(±)	1(±)
Fatty degeneration	0	0	0	0
Kidney				
Inflammatory cell infiltration, focal	1(±)	0	0	1(±)
Lung				
Inflammatory cell infiltration, focal	1(±)	1(±)	2(±)	1(±)

Table 6: *Histopathological findings of rats treated with RD-tapioca.*

n = 11/group; ± = minimal grade.

Based on the results, the NOAEL for RD-tapioca was determined to be 5,000 mg/kg bw/day, the highest dose tested, for both male and female rats.

Discussion

The mutagenicity and animal toxicity studies demonstrated the safety of resistant dextrin. The potential mutagenicity of RD-corn and RD-tapioca was evaluated in a bacterial reverse mutation test with *S. typhimurium* strains. RD-corn and RD-tapioca were not mutagenic up to 5,000 µg/plate in the absence and presence of metabolic activation. A mutagenicity study of an indigestible dextrin from potato starch demonstrated that doses up to 5,000 µg/plate were not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537, and *E. coli* WP2uvrA$^-$ in the absence and presence of metabolic activation [2]. For a dextrin produced from roasting wheat starch (Nutriose®FB or RD-wheat), doses up to 5,000 µg/plate were not mutagenic in *S. typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537 [3]. In addition, a hydrogenated resistant maltodextrin (Fibersol-2H) was not mutagenic at doses up to 5,000 µg/plate in the absence and presence of metabolic activation [4].

The acute toxicity of RD-corn and RD-tapioca was evaluated in SD rats by observing clinical signs for 14 days after a single oral administration. A transient hypoactivity and piloerection were found in both RD groups, but disappeared within 4 hours. No animals died. Therefore, the LD_{50} was determined to be above 20 g/kg bw for rats. Our findings are comparable to other research findings reported by other investigators. Wakabayashi., *et al*. [2] reported that the LD_{50} was higher than 20.0 g/kg bw for RD (indigestible dextrin) derived from potato starch in mice. An acute oral toxicity study of RD-wheat in fasted female SD rats found that the LD_{50} was more than 2,000 mg/kg bw [3]. A single oral administration of hydrogenated resistant maltodextrin determined the LD_{50} as more than 10 g/kg bw for SD rats [4]. Mucous, watery, or soft stool were observed at 5 and 10 g/kg bw Fibersol-2H doses, but these effects were mild and transient.

Our study found that the NOAEL for RD-tapioca was higher than 5,000 mg/kg bw/day for both male and female SD rats. From a 90-day toxicity study of RD-wheat, Wils., *et al*. [3] also reported that the NOAEL of RD-wheat was 5% in the diet, or 4,360 mg/kg bw/day for male SD rats and 6,500 mg/kg bw/day for female SD rats, the highest dose tested. In another 90-day toxicity study of hydrogenated resistant maltodextrin [4], the NOAEL was determined to be 5,000 mg/kg bw/day, the highest level tested. It is noteworthy that Fibersol 2H is a hydrogenated resistant dextrin prepared by hydrogenation of Fibersol-2, a soluble fiber preparation derived from cornstarch (https://www.fibersol.com/products/fibersol-2/), and that the terms, 'resistant maltodextrin' and 'resistant dextrin' are interchangeably used in the scientific community.

The present study did not find a caecal enlargement effect of RD-tapioca (data not shown), although this phenomenon was found in studies of RD-wheat and RD-hydrogenated corn [3,4]. In these studies, increased empty caecum weight was found in high dose groups. This change was considered a physiological adaptation to consumption of indigestible carbohydrates [5], as the components that are not completely digested and/or absorbed in the small intestine would result in an increased amount of osmotically active material in the intestinal contents, an increase in water retention (the animals tend to drink more), and finally, distension of the caecum to a size larger than normal.

Overall, resistant dextrins, regardless of their sources (tapioca, corn, or wheat), have NOAEL values ranging from 4,360 to 6,500 mg/kg bw/day in SD rats, and are considered safe as food ingredients [6].

Conclusion

Both RD-corn and RD-tapioca were not mutagenic and had LD_{50} values above 20 g/kg bw, the highest dose tested. From a 90-day oral toxicity study of RD-tapioca in SD rats, the NOAEL was 5,000 mg/kg bw/day for both male and female rats. Thus, RD-corn and RD-tapioca were considered safe as food ingredients.

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Conflict of Interest

The authors report that Steven Pracevic and Duanne Knapp are employed by Anderson Global Group (AGG), the sponsor of the study. However, all authors declare that their employment status may not be considered as potential competing interests. All the authors worked on this manuscript as volunteers without receiving any funding from any organization.

Author Contributions

Iris L. Case and Yunji Seol analyzed and interpreted the data to draft and update the manuscript. Steven Pracevic and Duanne Knapp secured the funds to sponsor this study and prepared the test substance, analyzed and interpreted the data, and decided to publish the data. Yonglin Gao, the corresponding author, organized the study plan, analyzed and interpreted the data, oversaw the entire study, and oversaw the study process.

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